they demonstrate that after gentamicin initiates the cell elimination process by targeting mitochondria, the result (cell death), after the 'point of no return' (mitochondrial permeabilization[2]) is passed, is realized through a number of different elements (enhanced ROS production, mitochondrial cytochrome *c* loss, NAD pool depletion) to guarantee the outcome. The mechanism by which metformin interferes with mitochondrial permeabilization in this case remains to be resolved; this understanding should facilitate the development of better ways to protect against unwanted cell death as a result of (unintended) mitochondrial injury.

DISCLOSURE
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REFERENCES

1. Zorov DB, Krasnikov BF, Kuzminova AE *et al.* Mitochondria revisited. Alternative functions of mitochondria. *Biosci Rep* 1997; **17**: 507–520.
2. Kroemer G, Reed JC. Mitochondrial control of cell death. *Nat Med* 2000; **6**: 513–519.
3. Plotnikov EY, Kazachenko AV, Vyssokikh MY *et al.* The role of mitochondria in oxidative and nitrosative stress during ischemia/reperfusion in the rat kidney. *Kidney Int* 2007; **72**: 1493–1502.
4. Humes HD. Aminoglycoside nephrotoxicity. *Kidney Int* 1988; **33**: 900–911.
5. Morales AI, Detaille D, Prieto M *et al.* Metformin prevents experimental gentamicin-induced nephropathy by a mitochondria-dependent pathway. *Kidney Int* 2010; **77**: 861–869.
6. Owen MR, Doran E, Halestrap AP. Evidence that metformin exerts its anti-diabetic effects through inhibition of complex 1 of the mitochondrial respiratory chain. *Biochem J* 2000; **348**: 607–614.
7. Zorov DB, Filburn CR, Klotz LO *et al.* Reactive oxygen species (ROS)-induced ROS release: a new phenomenon accompanying induction of the mitochondrial permeability transition in cardiac myocytes. *J Exp Med* 2000; **192**: 1001–1014.
8. Zorov DB, Juhaszova M, Yaniv Y *et al.* Regulation and pharmacology of the mitochondrial permeability transition pore. *Cardiovasc Res* 2009; **83**: 213–225.
9. Sahlin K, Katz A. The content of NADH in rat skeletal muscle at rest and after cyanide poisoning. *Biochem J* 1986; **239**: 245–248.
10. Di Lisa F, Ziegler M. Pathophysiological relevance of mitochondria in NAD(+) metabolism. *FEBS Lett* 2001; **492**: 4–8.

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The riddle of the sphinx redux

James A. Shayman[1]

Understanding the mechanisms of glucocorticoid-mediated inhibition of inflammation has been challenging. This is particularly true with regard to the development of drugs that mimic the anti-inflammatory benefits of steroids while avoiding the untoward metabolic effects. Förster *et al.* report that the inhibition of stress-induced mesangial-cell apoptosis by dexamethasone is mediated by sphingosine-1-phosphate. These findings identify alternative pathways whereby the anti-inflammatory mechanisms of glucocorticoids can be probed.

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Glucocorticoids (GCs), introduced 60 years ago for the treatment of inflammatory and autoimmune disorders, remain a mainstay of therapy for various renal diseases. Indeed, the rate of mortality from systemic lupus erythematosus fell precipitously following the introduction of steroids in the early 1950s. Remarkably, these agents remain a primary therapy for lupus nephritis. Steroids are limited by the wide range of untoward effects, including Cushing's syndrome, dyslipidemia, hypertension, pancreatitis, immunosuppression, bone necrosis and osteoporosis, muscle atrophy, cataracts, and hypogonadism.[1] Consequently, the identification of GCs with greater selectivity and specificity has been a Holy Grail for many pharmacologists.

It is disappointing, then, that despite extensive efforts expended in pursuit of this goal, very limited progress has been made in the development of such agents. The absence of progress is understandable as greater insight has been gained in discerning the anti-inflammatory mechanisms of action of GCs. One of the greatest hurdles in the development of more selective anti-inflammatory agents lies in the GC receptor itself. The levels of the receptor protein are regulated in both tissue-specific and cell cycle-specific fashions. Additionally, several splice and translation variants of the protein have been identified.

There are three primary mechanisms of action of GCs:[2] direct cortisol–GC receptor DNA interactions; protein interference mechanisms secondary to the transcription of gene products that interact with the cortisol–GC receptor complex; and nongenomic pathways, that is, the interaction of GCs with membrane receptors and second messengers. Each of these mechanisms has been well studied in exploring the anti-inflammatory mechanisms of GCs. Examples of GC-mediated inflammatory mechanisms include the inhibition of prostaglandin production by the repression of cyclooxygenase-2; the induction of MAPK phosphatase I, leading to the dephosphorylation and inactivation of Jun N-terminal kinase; and the direct physical interaction of the cortisol–GC receptor complex with nuclear factor-κB. The last mechanism is particular important because nuclear factor-κB induces the transcription of cyclooxygenase-2, as well as several cytokines, chemokines, and cell adhesion molecules.

Therefore, there are many levels at which the anti-inflammatory effects of GCs can be understood, and many sites at which GC homologs could be designed to increase specificity and potency. Examples

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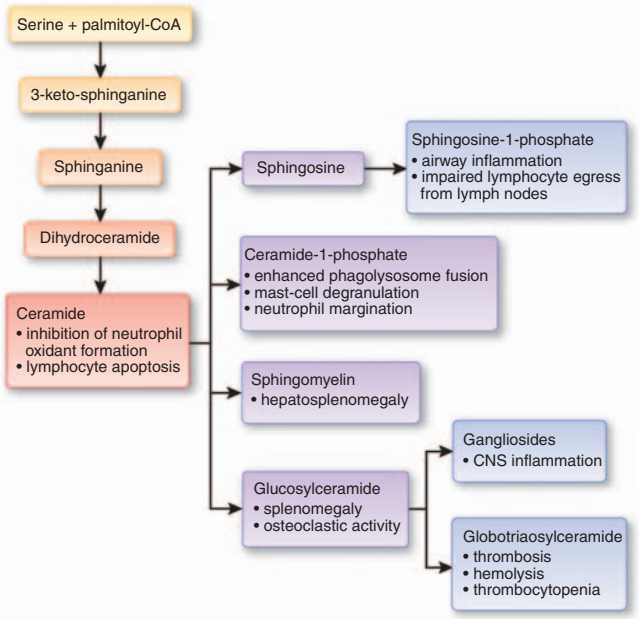

Figure 1 | Simple anabolic pathways governing sphingolipid formation and major bioactive sphingolipids. Bulleted items are examples of associations between major sphingolipids and inflammatory conditions that characterize classic clinical disorders. These disorders include Gaucher's disease (glucosylceramide); Fabry's disease and hemolytic uremic syndrome (globotriaosylceramide); Niemann–Pick disease (sphingomyelin); Farber's disease (ceramide); and brown recluse spider bites (ceramide-1-phosphate). CNS, central nervous system.

include the targeting of receptor–ligand binding and the inhibition of transactivation or transrepression. Inhibitors of transactivation would in theory target GC receptor-mediated upregulated genes, of which only a limited number have been identified. Transrepression appears to be the more common mechanism and involves the interference of the receptor with DNA-bound transcription factors. These factors include CREB, nuclear factor-κB, NFAT, STAT, and IRF3. These transcription factors in turn regulate the transcription of a large number of inflammatory proteins, including interleukin (IL)-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-12, IL-18, cyclooxygenase-2, E-selectin, inducible nitric oxide synthase, interferon-γ, tumor necrosis factor-α, intercellular adhesion molecule, vascular cell adhesion molecule, and monocyte chemoattractant protein-1. A number of these mediators have been considered as targets for novel therapeutics, typically in the form of antibodies. As an alternative strategy, one might explore whether all of the downstream mediators of GC actions have been identified and characterized.

Förster and colleagues[3] (this issue) have posed that question. They report that the induction of apoptosis in mesangial cells is rescued by treatment with dexamethasone and that the protective effect of dexamethasone occurs through the formation of sphingosine-1-phosphate. In support of this observation, they observed that dexamethasone upregulated the transcription of the precursor enzymes sphingosine kinase and neutral ceramidase. This effect was blocked in the presence of a sphingosine kinase inhibitor or in cells lacking a functional sphingosine kinase. These experiments focused on a simple but fundamental pathway of sphingolipid metabolism: the metabolism of ceramide by the neutral ceramidase to form sphingosine and sphingosine-1-phosphate. Because sphingosine is formed only from ceramide, the pathways coupling the ceramidase and sphingosine kinase are necessarily linked. These findings are significant because they identify sphingolipid signaling molecules as potential mediators of some of the anti-inflammatory effects of GCs. Sphingolipids are a class of lipids characterized by the presence of a long-chain lipophilic amine. These lipids were named by their discoverer, J.L.W. Thudicum, in reference to the riddle of the sphinx because of their enigmatic nature. The implication of the work of Förster *et al.*[3] is that the targeting of sphingolipid metabolites specifically may result in anti-inflammatory benefits with the avoidance of untoward side effects seen with GCs. Support for this strategy can be found in a large number of studies implicating specific sphingolipids as mediators of immune function. These include not only ceramide and sphingosine-1-phosphate, but ceramide-1-phosphate, free long-chain bases such as sphingosine, and a large number of ceramide-based glycosphingolipids (Figure 1).

However, establishing the merits of this strategy will require significant effort. First, because ceramide and sphingosine-1-phosphate are not the only sphingolipids implicated in signaling and other cellular functions, proof-of-principle experiments designed to establish a link between the anti-inflammatory effects of GCs and changes in either ceramide or sphingosine-1-phosphate will need to more comprehensively account for other bioactive sphingolipids. The flux of sphingolipids through the myriad of anabolic and catabolic pathways is rapid and dependent on multiple factors.[4] These include substrate availability, subcellular localization, lipid recycling, and feedback inhibition of synthetic enzymes, to name but a few. Second, the action of candidate bioactive sphingolipids will need to be better understood. Sphingosine-1-phosphate, for example, acts within cells as a second messenger and extracellularly as a ligand for one of several sphingosine-1-phosphate receptors.[5] Despite more than 20 years of work, the cellular targets for ceramide remain less well established, but include, for example, protein phosphatase 2A. Inhibition of this enzyme has been demonstrated to have secondary effects on a variety of receptor tyrosine kinases. Third, because the effects of GCs on proliferation and apoptosis are cell specific, it will be important to determine whether the sphingolipid effects are cell specific as well. Interestingly, ceramide has been reported to have both proapoptotic and pro-proliferative effects. Whether this is coincidental or reflects a biological nexus between GCs and ceramide should be actively explored.

Nevertheless, both sphingolipid mimetics and small-molecule inhibitors of sphingolipid-metabolizing enzymes have already been developed, lending credence to the view that these are 'druggable' pathways. These include FTY720 (fingolimod) and Genz-112638 (eliglustat). FTY720 is a structural homolog of myriocin, a fungal product and sphingosine analog. FTY720 is phosphorylated *in vivo* by sphingosine kinase 2 and subsequently binds to sphingosine-1-phosphate receptors or inhibits intracellular targets such as cPLA2 and ceramide synthase. The immunosuppressive effects of FTY720 are believed to be secondary to the prevention of the egress of lymphocytes from lymph nodes.[6] FTY720 has been reported to reduce the frequency of relapses in multiple sclerosis. A regulatory filing seeking approval for the treatment of multiple sclerosis was recently announced. The development of FTY720 was halted when the compound was found to be no more effective than conventional therapy in the prevention of organ rejection. Genz-112638 is a highly selective inhibitor of glucosylceramide synthase. This compound blocks the glycosylation of ceramide to form glucosylceramide, the base cerebroside for more than 80% of mammalian glycosphingolipids. Recent phase 2 trials of the use of Genz-112638 for the treatment of type I Gaucher's disease have reported that this oral compound is comparable to enzyme replacement therapy in the reduction of spleen size and the improvement of anemia and thrombocytopenia.[7] Phase 3 trials are currently in progress. Proof-of-concept studies have also been conducted with this class of compounds in mouse models of Fabry's disease, suggesting that inhibition of glycosphingolipids with Genz-112638, including globotriaosylceramide, will also provide an alternative treatment strategy for this renal disease.

Thus sphingolipid biology offers a new window that may not only shed light on the old problem of dissociating the anti-inflammatory effects of GC from its secondary metabolic actions, but may offer new strategies for the development of novel therapeutics. Although it remains to be proven whether specific sphingolipids mediate inflammatory and autoimmune diseases of the kidney, these metabolites have already been demonstrated to mediate inflammatory and proliferative responses in a variety of other organs and pathological settings. For clinicians and investigators focused on renal inflammatory disease, these pathways pose new riddles to be solved.

DISCLOSURE
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REFERENCES

1. Rhen T, Cidlowski JA. Antiinflammatory action of glucocorticoids: new mechanisms for old drugs. *N Engl J Med* 2005; **353**: 1711–1723.
2. De Bosscher K, Haegeman G. Minireview: latest perspectives on antiinflammatory actions of glucocorticoids. *Mol Endocrinol* 2009; **23**: 281–291.
3. Förster A, Emmler T, Schwalm S *et al.* Glucocorticoids protect renal mesangial cells from apoptosis by increasing cellular sphingosine-1-phosphate. *Kidney Int* 2010; **77**: 870–879.
4. Shayman JA. Sphingolipids. *Kidney Int* 2000; **58**: 11–26.
5. Bandhuvula P, Saba JD. Sphingosine-1-phosphate lyase in immunity and cancer: silencing the siren. *Trends Mol Med* 2007; **13**: 210–217.
6. Japtok L, Kleuser B. The role of sphingosine-1-phosphate receptor modulators in the prevention of transplant rejection and autoimmune diseases. *Curr Opin Investig Drugs* 2009; **10**: 1183–1194.
7. Peterschmitt J, Lukina E, Watman N *et al.* Genz-112638, an investigational oral treatment for Gaucher disease type 1: preliminary phase 2 clinical trial results. *Mol Genet Metab* 2009; **96**: S34[abstr].

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Ironing out the phosphorus problem

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Control of serum phosphorus remains a vexing problem in chronic kidney disease. Although novel dialysis regimens may provide excellent phosphorus control, phosphate binders remain necessary for most dialysis patients. Block *et al.* present a phase I clinical trial examining the safety and efficacy of SBR759, a novel non-calcium, iron-based phosphate binder. Although the risks of iron accumulation and hypocalcemia must be addressed, this phosphate binder appears to be well tolerated and effective and offers a powder-based formulation.

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Hyperphosphatemia has been linked with increased mortality and cardiovascular disease in chronic kidney disease (CKD). Although control of serum phosphorus levels can be obtained with prolonged nocturnal dialysis by itself,[1] the vast majority of patients receiving currently recommended dialysis regimens require the use of phosphate-binding agents.

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The ideal phosphate binder has yet to be identified, and today there are multiple options for prescribing such agents. Nearly 40 years ago, aluminum-based binders were introduced, and although effective, chronic administration led to significant tissue accumulation and to the development of encephalopathy, osteomalacia, myopathy, and microcytic anemia. Such compounds were replaced by calcium-based binders, which are both effective and inexpensive. However, the success of calcium-based binders has been tempered by evidence that their use leads to higher serum calcium levels, more frequent episodes of hypercalcemia, adynamic bone disease, diminished bone buffer capacity, and vascular calcifications.[2–4]